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(71) Applicant *(for all designated States except US)*: THE PENN STATE RESEARCH FOUNDATION [US/US]; 114 Kern Graduate Building, University Park, PA 16802 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ARTECA, Richard, N. [US/US]; 455 Westgate Drive, State College, PA 16803 (US). WICKREMESINHE, Enaksha [LK/US]; 6H Graduate Circle, State College, PA 16801 (US).

(74) Agent: MONAHAN, Thomas, J.; Intellectual Property Office, The Pennsylvania State University, 114 Barbara Building II, University Park, PA 16802 (US).

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(54) Title: CULTURED *TAXUS* TISSUES AS A SOURCE OF TAXOL, RELATED TAXANES AND OTHER NOVEL ANTI-TUMOR/ANTI-VIRAL COMPOUNDS

(57) Abstract

Successful culture methods have been developed which result in stable, long-term tissue cultures derived from *Taxus* explants and hydroponically grown roots. These cultures offer a rapidly reproducible, continuously-available source for the production of purified taxol and taxol-related compounds. Culture methods include *in vitro* tissue culture and hydroponics. Cultures are initiated with stem or root tissues of *Taxus* or from roots grown hydroponically. Taxol production may be scaled to commercial levels by use of bioreactors. Screening assays are provided for species and cultures of *Taxus* that are sources of taxol and taxol-related compounds. In addition to obtaining the same compositions as presently directly extracted from yew trees, new compositions exhibiting taxol-like activity, have been purified from the novel *Taxus* sources, offering new horizons for chemotherapeutic agent development.

What Is Claimed Is:

1. A method for producing a purified compound selected from the group consisting of taxol, a precursor or intermediate of the biochemical pathway resulting in taxol production, and a derivative of taxol, said method comprising:

(a) culturing a tissue explant from a plant of the genus *Taxus* under conditions which allow formation of a culture and production of the compound by the cultured cells;
and

(b) collecting the compound from the culture or from the conditioned medium of the culture.

2. The method of claim 1, wherein the tissue comprises a root or a young stem.

3. The method of claim 2, where the root is derived from a dormant plant.

4. The method of claim 1, further comprising enhancing production of secondary metabolites of the compounds by applying elicitation techniques during culturing.

5. The method of claim 3, wherein elicitation techniques comprise feeding taxol precursors to the culture.

6. The method of claim 5, wherein the taxol precursor is acetate.

7. The method of claim 4, wherein elicitation techniques comprise adding fungal extracts.

8. The method of claim 1, comprising further isolating and purifying of the collected compound to remove non-taxol-containing compounds and contaminants.

9. The method of claim 2, wherein the root is a hairy root.

10. The method of claim 9, wherein the hairy root is produced by infection of a non-hairy *Taxus* root with *Agrobacterium*.

11. The method of claim 1, wherein the culturing is by means of *in vitro* tissue culture of a root.

12. The method of claim 1, wherein the culturing is by means of hydroponic growth of roots.

13. The method of claim 1, wherein the compound is 9-dihydro-13-acetyl baccatin-3, and the plant of the genus *Taxus* is *Taxus canadensis*.

14. The method of claim 1, wherein *Taxus* is further defined as *Taxus media cv*. Hicksii.

15. The method of claim 1, wherein the compound is capable of inhibiting cell growth in a cancer model system or of producing positive results in a microtubule-stabilizing bioassay.

16. The method of claim 15, wherein the cancer model system comprises a cell line selected from a group consisting of B16 melanoma, MX-1 mammary xenograft, P388 leukemia, KB, and L1210 leukemia.

17. A method for establishing a cell line from a culture of *Taxus*, said method comprising the steps of:

(a) selecting a tissue source for an explant from the group consisting of a young stem and a root;

(b) culturing the explant in callus-inducing medium to form a callus;

(c) subculturing selected sections of the callus to promote subclone growth;

(d) selecting a subclone to develop a cell line; and

(e) culturing the cell line on a solid support in maintenance medium.

18. The method of claim 17, wherein *Taxus* is further defined as *Taxus media* cv. Hicksii.

19. The method of claim 17, wherein the callus-inducing medium comprises Gamborg's B5 medium supplemented with plant hormones.

20. The method of claim 19, wherein the plant hormones are selected from a group consisting of 2,4-D, kinetin and a combination of 2,4-D and kinetin.

21. The method of claim 17, wherein the selected sections of callus are selected based on criteria selected from the group consisting of high growth rate compared to growth rate of other callus, lack of red

coloring, said coloring indicating the presence of exudates which inhibit growth, presence of yellow coloring, and friability.

22. The method of claim 17, wherein the solid support for maintenance of the cell line comprises a membrane raft and the maintenance medium comprises modified Gamborg's B5 medium according to Table IV.

23. The method of claim 17, wherein the maintenance medium comprises casein hydrolysate, glucose and fructose.

24. The method of claim 23, wherein the concentration of glucose and fructose totals about 10-40 g/l.

25. The method of claim 17, wherein the maintenance medium comprises a sugar selected from a group consisting of sucrose, glucose and fructose, and wherein the concentration of said sugar is about 40-80 g/l.

26. The method of claim 23, wherein the concentration of casein hydrolysate is about 0.2-1.0 g/l.

27. The method of claim 17, wherein the root is derived from a plant in the dormant phase.

28. A purified compound that is the product of a process according to claim 1, wherein said compound is selected from the group consisting of taxol, a precursor or intermediate of the biochemical pathway resulting in taxol production, and a derivative of taxol.

29. A cell line, derived from a *Taxus* tissue, said cell line having the following characteristics:

(a) stable, long term growth in culture; and

(b) capability of expressing a compound selected from the group consisting of taxol, a precursor or intermediate of the biochemical pathway resulting in the production of taxol, and a derivative of taxol.

30. The cell line of claim 29, wherein *Taxus* comprises *Taxus media* cv. Hicksii.

31. The cell line of claim 29, wherein the compound is capable of inhibiting cell growth in cancer model systems or of producing positive results in a microtubule-stabilizing bioassay.

32. The cell line of claim 31, wherein the cancer model system comprises a cell line selected from a group consisting of B16 melanoma, MX-1 mammary xenograft, P388 leukemia, KB, and L1210 leukemia.

33. The cell line of claim 29, further defined as producing an amount of taxol that is at least about 1-2 fold increased above that produced by direct extraction from stems, leaves or bark of *Taxus media* cv. Hicksii.

34. A method of producing taxol, said method comprising:

(a) placing a cell line or a hydroponic root culture derived from *Taxus* in a bioreactor;

(b) operating the bioreactor so that taxol is produced by the cell line or root and is secreted into the nutrient medium of the bioreactor; and

(c) collecting the taxol from the medium.

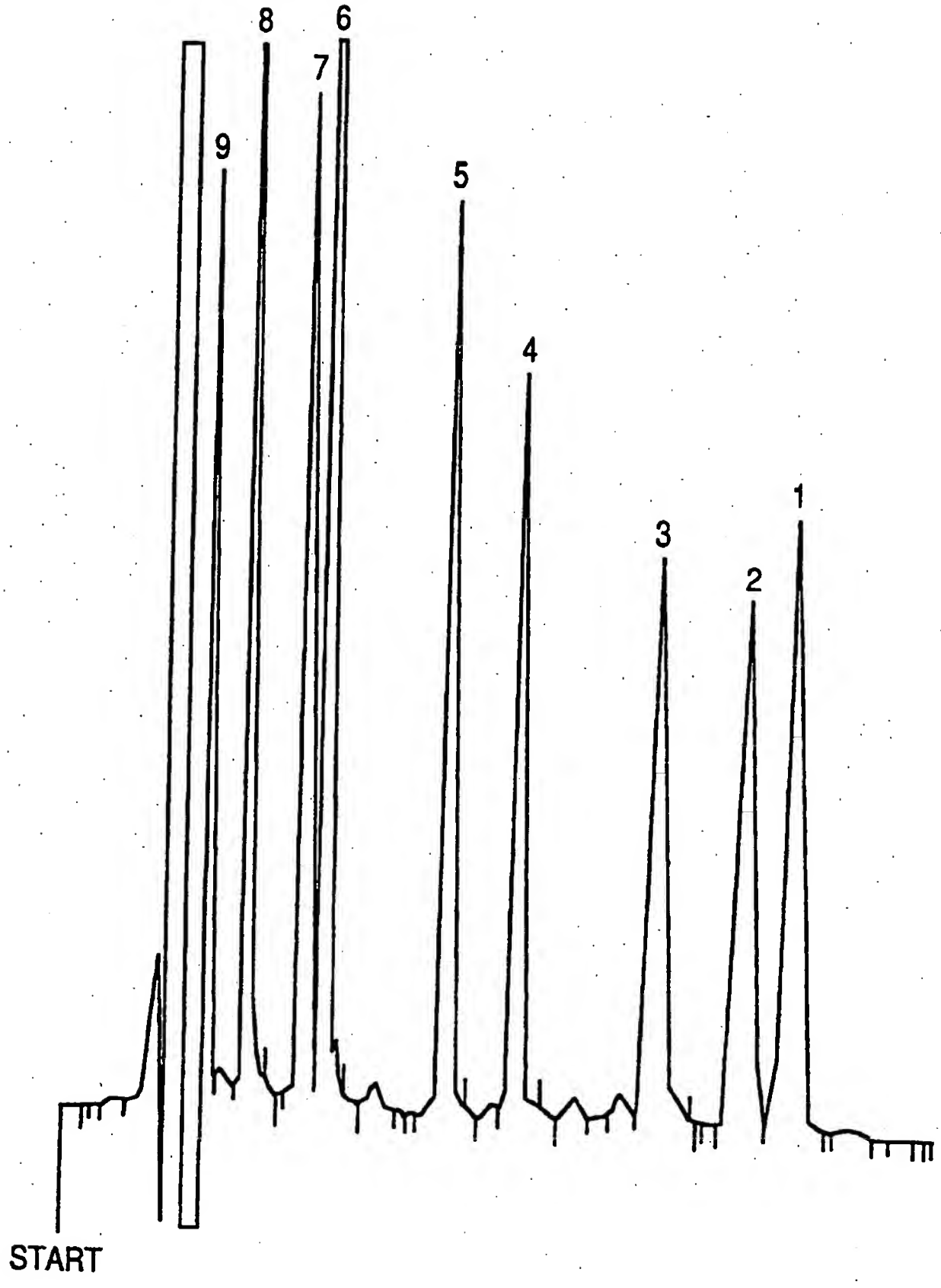
35. The method of claim 34, wherein the cell line is produced according to claim 17.

36. A compound exhibiting microtubule-stabilizing activity, wherein said compound is selected from the group consisting of peaks eluting on an HPLC profile from a Curosil G column, number 40, of an extract from a stable *Taxus* cell line or a hydroponically grown root.

37. The compound of claim 36, wherein the *Taxus* cell line is CR-1 and the peak selected elutes at 31 minutes.

38. The compound of claim 36, selected from a group consisting of peaks A-K according to FIGURES 3-5.

FIG. 1



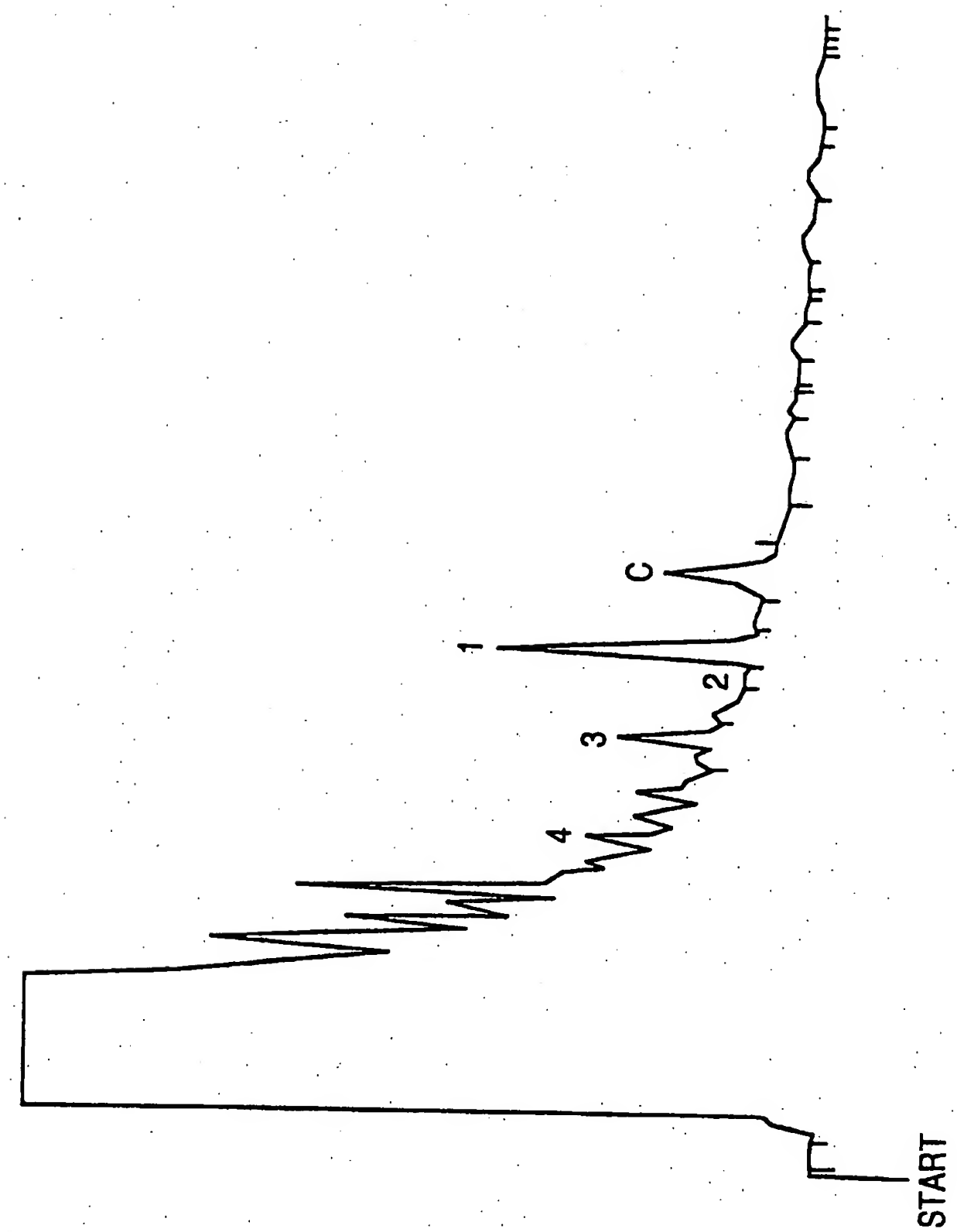


FIG. 2

FIG. 3